

Table 1. Demographic characteristics of the study population	
Age (years)	Mean (SD)
18-24	20.5 (2.5)
25-34	29.5 (4.5)
35-44	39.5 (5.5)
45-54	49.5 (6.5)
55-64	59.5 (7.5)
65-74	69.5 (8.5)
75-84	79.5 (9.5)
85-94	89.5 (10.5)
95-104	99.5 (11.5)
105-114	109.5 (12.5)
115-124	119.5 (13.5)
125-134	129.5 (14.5)
135-144	139.5 (15.5)
145-154	149.5 (16.5)
155-164	159.5 (17.5)
165-174	169.5 (18.5)
175-184	179.5 (19.5)
185-194	189.5 (20.5)
195-204	199.5 (21.5)
205-214	209.5 (22.5)
215-224	219.5 (23.5)
225-234	229.5 (24.5)
235-244	239.5 (25.5)
245-254	249.5 (26.5)
255-264	259.5 (27.5)
265-274	269.5 (28.5)
275-284	279.5 (29.5)
285-294	289.5 (30.5)
295-304	299.5 (31.5)
305-314	309.5 (32.5)
315-324	319.5 (33.5)
325-334	329.5 (34.5)
335-344	339.5 (35.5)
345-354	349.5 (36.5)
355-364	359.5 (37.5)
365-374	369.5 (38.5)
375-384	379.5 (39.5)
385-394	389.5 (40.5)
395-404	399.5 (41.5)
405-414	409.5 (42.5)
415-424	419.5 (43.5)
425-434	429.5 (44.5)
435-444	439.5 (45.5)
445-454	449.5 (46.5)
455-464	459.5 (47.5)
465-474	469.5 (48.5)
475-484	479.5 (49.5)
485-494	489.5 (50.5)
495-504	499.5 (51.5)
505-514	509.5 (52.5)
515-524	519.5 (53.5)
525-534	529.5 (54.5)
535-544	539.5 (55.5)
545-554	549.5 (56.5)
555-564	559.5 (57.5)
565-574	569.5 (58.5)
575-584	579.5 (59.5)
585-594	589.5 (60.5)
595-604	599.5 (61.5)
605-614	609.5 (62.5)
615-624	619.5 (63.5)
625-634	629.5 (64.5)
635-644	639.5 (65.5)
645-654	649.5 (66.5)
655-664	659.5 (67.5)
665-674	669.5 (68.5)
675-684	679.5 (69.5)
685-694	689.5 (70.5)
695-704	699.5 (71.5)
705-714	709.5 (72.5)
715-724	719.5 (73.5)
725-734	729.5 (74.5)
735-744	739.5 (75.5)
745-754	749.5 (76.5)
755-764	759.5 (77.5)
765-774	769.5 (78.5)
775-784	779.5 (79.5)
785-794	789.5 (80.5)
795-804	799.5 (81.5)
805-814	809.5 (82.5)
815-824	819.5 (83.5)
825-834	829.5 (84.5)
835-844	839.5 (85.5)
845-854	849.5 (86.5)
855-864	859.5 (87.5)
865-874	869.5 (88.5)
875-884	879.5 (89.5)
885-894	889.5 (90.5)
895-904	899.5 (91.5)
905-914	909.5 (92.5)
915-924	919.5 (93.5)
925-934	929.5 (94.5)
935-944	939.5 (95.5)
945-954	949.5 (96.5)
955-964	959.5 (97.5)
965-974	969.5 (98.5)
975-984	979.5 (99.5)
985-994	989.5 (100.5)
995-1004	999.5 (101.5)
1005-1014	1009.5 (102.5)
1015-1024	1019.5 (103.5)
1025-1034	1029.5 (104.5)
1035-1044	1039.5 (105.5)
1045-1054	1049.5 (106.5)
1055-1064	1059.5 (107.5)
1065-1074	1069.5 (108.5)
1075-1084	1079.5 (109.5)
1085-1094	1089.5 (110.5)
1095-1104	1099.5 (111.5)
1105-1114	1109.5 (112.5)
1115-1124	1119.5 (113.5)
1125-1134	

08-27-00

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PTO/SB/05 (4/98)
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**UTILITY
PATENT APPLICATION
TRANSMITTAL**

(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))

Attorney Docket No. UF-243X

First Inventor or Application Identifier	Howard M. Johnson
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Title	Materials and Methods for Inhibition of IgE Production
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Express Mail Label No.	EK318904486US
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APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

ADDRESS TO: Assistant Commissioner for Patents
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1. ☒ * Fee Transmittal Form (e.g., PTO/SB/17)
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2. ☒ Specification [Total Pages 27]
(preferred arrangement set forth below)
- Descriptive title of the Invention
 - Cross References to Related Applications
 - Statement Regarding Fed sponsored R & D
 - Reference to Microfiche Appendix
 - Background of the Invention
 - Brief Summary of the Invention
 - Brief Description of the Drawings (if filed)
 - Detailed Description
 - Claim(s)
 - Abstract of the Disclosure
3. ☒ Drawing(s) (35 U.S.C. 113) [Total Sheets 7]
4. Oath or Declaration [Total Pages 2]

5. ☐ Microfiche Computer Program (*Appendix*)
6. Nucleotide and/or Amino Acid Sequence Submission
(*if applicable, all necessary*)
- a. ☐ Computer Readable Copy
- b. ☐ Paper Copy (identical to computer copy)
- c. ☐ Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

7. ☐ Assignment Papers (cover sheet & document(s))
8. ☐ 37 C.F.R. § 3.73(b) Statement ☐ Power of Attorney
(when there is an assignee)
9. ☐ English Translation Document (if applicable)
10. ☐ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS Citations
11. ☐ Preliminary Amendment
12. ☒ Return Receipt Postcard (MPEP 503)
(Should be specifically itemized)
- * Small Entity
13. ☒ Statement(s) ☐ Statement filed in prior application, Status still proper and desired
(PTO/SB/09-12)
14. ☐ Certified Copy of Priority Document(s)
(if foreign priority is claimed)
15. ☒ Other: Claims priority to

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USSN 60/151,026, filed
August 27, 1999

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- | | | | |
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|---------------------------------------|-------------------------------------|---|--|

Prior application information: Examiner _____ Group / Art Unit: _____

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17. CORRESPONDENCE ADDRESS

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Signature		Date	Aug. 25, 2000

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DOCKET NO. UF-243X**FEE TRANSMITTAL FORM**

Box PATENT APPLICATION
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 Washington, D.C. 20231

Sir:

Transmitted herewith for filing is the patent application of:


Inventor(s): Howard M. Johnson, Mustafa G. MujtabaEntitled: Materials and Methods for Inhibition of IgE Production

- ☒ A Utility Patent Application Transmittal Form accompanies this Fee Transmittal Form.
☒ The Filing Fee is calculated below:

CLAIMS AS FILED				
	Number filed	Number Extra	Rate	Fee
Basic Fee			\$345.00	\$345.00
Total Claims	24 - 20 =	4	x \$9	\$ 36.00
Independent Claims	4 - 3 =	1	x \$39	\$ 39.00
Presentation of Multiple Dependent Claim(s) (\$130)				\$
Total Filing Fee				\$420.00

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August 25, 2000
 Date



 Attorney of Record


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005680-19884950

Applicant or Patentee: Howard M. Johnson, Mustafa G. Mujtaba Attorney's
Serial or Patent No.: _____ Docket No. UF-243X
Filed or Issued: August 25, 2000
For: Materials and Methods for Inhibition of IgE Production

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY
STATUS (37 CFR 1.9 (f) and 1.27 (c)) – NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION University of Florida
ADDRESS OF ORGANIZATION 223 Grinter Hall
Gainesville, FL 32611

TYPE OF ORGANIZATION

- ☒ UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION
☐ TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501(a)(3))
☐ NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA
(NAME OF STATE _____)
(CITATION OF STATUTE _____)
☐ WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501(a) and 501(c)(3)) IF LOCATED IN THE UNITED STATES OF AMERICA
☐ WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA
(NAME OF STATE _____)
(CITATION OF STATUTE _____)

I hereby declare that the above identified nonprofit organization qualifies as a nonprofit organization as defined in 37 CFR 1.9 (d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, with regard to the invention described in the above-identified:

☐ PATENT ☒ APPLICATION

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization identified above with regard to the above-identified invention.

If the rights held by the above identified nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9 (d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9 (d) or a nonprofit organization under 37 CFR 1.9 (e).

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring their status as small entities. (37 CFR 1.27)

NAME _____
ADDRESS _____
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

NAME _____
ADDRESS _____
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change of status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28 (b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Thomas E. Walsh, Ph.D.
TITLE IN ORGANIZATION Director of Sponsored Research
ADDRESS OF PERSON SIGNING 223 Grinter Hall
Gainesville, FL 32611

SIGNATURE Thomas E. Walsh DATE August 25, 2000

DESCRIPTION

MATERIALS AND METHODS FOR INHIBITION OF IgE PRODUCTION

5 The subject invention was made with government support under a research project supported by National Institute of Health Grant No. CA69959 and R37AI25904. The government has certain rights in this invention.

Cross-Reference to a Related Application

10 This application claims the benefit of U.S. Provisional Application No. 60/151,026, filed August 27, 1999.

Background of the Invention

15 The interferons have been classified into two distinct groups: type I interferons, including IFN α , IFN β , and IFN ω (also known as IFN α II); and type II interferons, represented by IFN γ (reviewed by DeMaeyer *et al.*, 1998). In humans, it is estimated that there are at least 17 IFN α non-allelic genes, at least about 2 or 3 IFN β non-allelic genes, and a single IFN γ gene.

20 IFN α 's have been shown to inhibit various types of cellular proliferation. IFN α 's are especially useful against hematologic malignancies such as hairy-cell leukemia (Quesada *et al.*, 1984). Further, these proteins have also shown activity against multiple myeloma, chronic lymphocytic leukemia, low-grade lymphoma, Kaposi's sarcoma, chronic myelogenous leukemia, renal-cell carcinoma, urinary bladder tumors and ovarian cancers (Bonnem *et al.*, 1984; Oldham, 1985). The role of interferons and interferon receptors in the
25 pathogenesis of certain autoimmune and inflammatory diseases has also investigated (Benoit *et al.*, 1993).

 IFN α 's are also useful against various types of viral infections (Finter *et al.*, 1991). Alpha interferons have shown activity against human papillomavirus infection, Hepatitis B,

and Hepatitis C infections (Finter *et al.*, 1991; Kashima *et al.*, 1988; Dusheiko *et al.*, 1986; Davis *et al.*, 1989). In addition, studies with IFN α and IFN γ have shown suppression of IgE production in allergic diseases (Noh *et al.*, 1998; Hofstra *et al.*, 1998; Lack *et al.*, 1996; Dolen *et al.*, 1995; Kimata *et al.*, 1995; Gruschwitz *et al.*, 1993).

5 Significantly, however, the usefulness of IFN α 's has been limited by their toxicity: use of interferons in the treatment of cancer and viral disease has resulted in serious side effects, such as fever, chills, anorexia, weight loss, and fatigue (Pontzer *et al.*, 1991; Oldham, 1985). These side effects often require (i) the interferon dosage to be reduced to levels that limit the effectiveness of treatment, or (ii) the removal of the patient from treatment. Such toxicity has reduced the usefulness of these potent antiviral and antiproliferative proteins in
10 the treatment of debilitating human and animal diseases.

Interferon-tau (IFN τ) is a member of the type I IFN family but, unlike IFN α and IFN β , IFN τ lacks toxicity at high concentrations *in vitro* and when used *in vivo* in animal studies (Bazer *et al.*, 1989; Pontzer *et al.*, 1991; Soos, Johnson, 1995; Soos, *et al.*, 1995; Soos *et al.*, 1997; Khan *et al.*, 1998). IFN τ was originally identified as a pregnancy
15 recognition hormone produced by trophoblasts cells of the placenta of ruminants such as sheep and cows (Bazer *et al.*, 1991; Godkin *et al.*, 1982; Imakawa *et al.*, 1987; Johnson *et al.*, 1994). It has been reported that a human IFN τ exists (Whaley *et al.*, 1994) but this observation has not been confirmed. Thus, it is currently unknown as to whether there is a
20 human IFN τ . IFN τ exhibits antiviral and cell inhibitory properties are very similar to that of IFN α and IFN β (Bazer *et al.*, 1989; Pontzer *et al.*, 1991; Soos, Johnson, 1995). However, IFN τ lacks the cellular toxicity associated with high concentrations of IFN α and IFN β (Bazer *et al.*, 1989; Pontzer *et al.*, 1991). Further, the weight loss and bone marrow suppression that is associated with administering high doses of IFN α and IFN β to individuals
25 is absent with IFN τ in animal systems (Soos, Johnson, 1995; Soos *et al.*, 1995; Soos *et al.*, 1997). Studies have shown that the N-terminus of type I IFNs play a role in the toxicity or lack thereof for an IFN (Pontzer *et al.*, 1994; Subramaniam *et al.*, 1995).

It has been reported that IFN τ suppresses the humoral and cellular responses in experimental allergic encephalomyelitis (EAE), an animal model for the autoimmune disease, multiple sclerosis (Mujtaba *et al.*, 1998). It has been shown that IFN τ suppresses the responses of lymphocytes to mitogens such as Con A and superantigens such as SEA and SEB (Soos, Johnson, 1995; Soos *et al.*, 1995; Khan *et al.*, 1998). There are also reports, again in the EAE model, that IFN τ and other type I IFNs can induce IL-10 and TGF β , but not IL-4, production by cells that have already been activated by antigen presenting cells (Soos, Subramaniam *et al.*, 1995; Mujtaba *et al.*, 1998; Mujtaba *et al.*, 1997). IFN τ has also been suggested for use in the treatment of Multiple Sclerosis in humans.

Production of IgE immunoglobulin is important in mediating allergic diseases such as allergic rhinitis, atopic dermatitis, bronchial asthma, and food allergy. Allergic sensitization of mice by intraperitoneal (ip) injection with ovalbumin (OVA) as an allergen and aluminum hydroxide as an adjuvant is a well characterized method of stimulating IgE production *in vivo* (Mancino *et al.*, 1980; Miguel *et al.*, 1977; Beck *et al.*, 1989). When OVA-immunized mice are challenged with aerosolized OVA, they show inflammatory cell infiltration in the submucosal layer of the lungs (Kay *et al.*, 1992; Hamelmann *et al.*, 1996; Hamelmann *et al.*, 1997). IgE can stimulate the release of certain chemotactic mediators from mast cells that can lead to active accumulation of macrophages and granulocytes at the site. Also, production of a further set of inflammatory molecules by these cells can lead to allergic asthma. Thus, allergen specific IgE production by B cells is important in the pathogenesis of allergic diseases.

Conventional therapy for allergic disease consists of decongestants and anti-histamines, which function to reduce symptoms after allergic responses occur. Thus, there remains a need in the art for a treatment of allergic diseases which lacks toxic side effects and functions to block allergic response, thus acting prior to symptomology.

Brief Summary of the Invention

The subject invention concerns novel methods and materials for treating patients afflicted with allergic conditions, such as allergic rhinitis, atopic dermatitis, bronchial asthma and food allergy. The method of the subject invention comprises administering a type I interferon, such as interferon tau (IFN τ), or a chimeric IFN (for example, ovine IFN τ (1-27)/human IFN α D (28-166)) to a person afflicted with an allergic condition. When administered, the interferon suppresses the production of allergen-specific IgE antibodies without toxic side effects. The subject invention also concerns chimeric ovine/human IFNs which can be used in the methods of the invention.

Brief Description of the Drawings

Figures 1A and 1B show the inhibition of OVA-specific IgE antibody production in OVA-sensitized mice by IFN τ treatment. BALB/C mice were immunized by ip injection with ovalbumin (OVA) mixed with aluminum hydroxide and boosted seven days later. The mice were exposed to aerosolized OVA (1% w/v) on days 19 and 20 after immunization for 20 minutes. Mice were treated daily with ip injections of IFN τ (5×10^5 U/day) or PBS starting three days prior to immunization. Blood was collected 24 h prior to (Figure 1A) and 24 h after (Figure 1B) aerosolized OVA exposure. Direct ELISA was performed to detect OVA-specific IgE levels. Two to three mice per group were used, and average absorbance is shown. Control absorbance using a normal mouse serum has been subtracted out from each dilution point. Statistical significance for the inhibition of OVA-specific IgE antibody production was shown by Student's *t* test at all dilutions (except for the 10,000 dilution) for IFN τ treatment as compared to PBS treatment ($p < 0.05$).

Figures 2A-2C show the histological evaluation of OVA-immunized mice after treatment with PBS or IFN τ . BALB/C mice were immunized with OVA and exposed to aerosolized OVA on day 20 after immunization and treated with PBS or IFN τ as previously described. Twenty-four hours after aerosolized OVA treatment, lungs from non-immunized (Figure 2A), PBS treated (Figure 2B), and IFN τ treated (Figure 2C) mice were extracted,

fixed, embedded in paraffin, sectioned and stained with hematoxylin and eosin for inflammatory cells. Arrows indicated the epithelium of the bronchiole.

Figure 3 shows IL-4 levels in sera of OVA-immunized mice treated with PBS or IFN τ . BALB/C mice were immunized with OVA and exposed to aerosolized OVA and treated with PBS or IFN τ as previously described in Figure 1 description. Blood was collected 24 h after aerosolized OVA exposure, and a sandwich ELISA for IL-4 was performed. Two to three mice per group were used, and average amount (ng) of IL-4 is shown. Control level from naive mouse serum was subtracted from the PBS and IFN τ levels.

Figures 4A and 4B show *in vivo* treatment of OVA-immunized mice with IFN τ reduces OVA-stimulation of splenocytes. BALB/C mice were immunized with OVA and exposed to aerosolized OVA and treated with PBS or IFN τ as previously described in the Figure 1 legend. Spleen cells (5×10^5 cells/well) were cultured with OVA at 100 μ g/ml for 72 h, after which the cultures were pulsed with tritiated thymidine. PBS-treated splenocytes were also incubated with BSA and MBP at 100 μ g/ml (Figure 4B). Cell associated radioactivity was quantified 12 h later using a β -scintillation counter, and data from one of three experiments are presented as mean cpm of quadruplicate wells \pm SD. Statistical significance for the inhibition of OVA-induced cell proliferation by IFN τ treatment as compared to PBS treatment was shown using Student's *t* test ($p < 0.001$).

Figure 5 shows *in vitro* treatment of splenocytes with type I IFNs inhibit OVA-specific proliferation. BALB/C mice were immunized by ip injection of OVA mixed with aluminum hydroxide and boosted 7 days later. The mice were exposed to aerosolized OVA (1% w/v) on day 19 and 20 for 20 minutes. Spleen cells (5×10^5 cells/well) were cultured with 15000 U/ml of various IFNs and media in the presence or absence of 100 μ g/ml OVA for 84 h, after which the cultures were pulsed with tritiated thymidine. Cell associated radioactivity was quantified 12 h later using a β -scintillation counter, and data from one of three experiments are presented as mean cpm of quadruplicate wells \pm SD. Inhibition of OVA specific splenocyte proliferation by all the IFNs was statistically significant as

compared to OVA-specific splenocyte proliferation of OVA-sensitized medium-treated cells as shown by Student's *t* test ($p < 0.001$).

Figures 6A-6C show immunoblot detection of mouse and human IgE in culture supernatants taken from ovalbumin (OVA)-sensitized mouse splenocytes or human myeloma B cells treated with various IFNs or media. Figure 6A includes Lane 1, control mouse IgE; lane 2, RPMI 1640 supplemented with 10% FBS; lanes 3 and 4, 84 h supernatants from naive mouse splenocytes cultured in the absence or presence of OVA, respectively; lanes 5 and 6, 84 h supernatants from PBS-treated OVA-sensitized mouse splenocytes cultured in the absence or presence of OVA, respectively; lanes 7 and 8, 84 h supernatant from IFN τ -treated OVA-sensitized mouse splenocytes in absence or presence of OVA, respectively. Figure 6B includes Lane 1, control mouse IgE; lane 2, RPMI 1640 medium only; lane 3, 84 h splenocyte culture in the absence of OVA; lanes 4, 5, 6, and 7, 84 h splenocyte (5×10^5 cells/well) cultures with OVA in presence of media, IFN τ , IFN τ /IFN α chimeric, and IFN α D, respectively. Figure 6C includes Lane 1, RPMI 1640 medium only; lanes 2, 3, 4, and 5, IgE producing U266BL cells, which were starved overnight, and incubated at 2×10^5 cells/well for 96 h in the presence of media, 1.0×10^4 U/ml IFN α D, IFN τ /IFN α D chimeric, and IFN τ , respectively.

Figure 7 shows the inhibition of proliferation of the IgE-producing human myeloma B cell line U266 by type I IFNs. The IgE-producing U266BL cells, which were starved overnight, were incubated at 2×10^5 cells/well in the presence of 1.0×10^4 U/ml of IFN τ , IFN τ /IFN α D chimeric, IFN α D, and media 72 h. Cultures pulsed with tritiated thymidine, and cell associated radioactivity was quantified 12 h later using a β -scintillation counter, and data from one of three experiments are presented as mean cpm of quadruplicate wells \pm SD. Percent cell viability, as measured by trypan blue exclusion test, is presented above each bar. Statistical significance for the inhibition of cell proliferation was shown by Student's *t* test for all the IFN treatments as compared to the media treatment ($p < 0.001$).

Figure 8 shows the metabolic activity of human peripheral blood mononuclear cells (HPBMC) after treatment with IFNs. HPBMC were cultured in the presence of varying

concentrations (250 to 100,000 U/ml) of IFN τ , IFN α D, and IFN τ /IFN α D chimeric for seven days. Metabolic activity of HPBMC was assessed by measuring cell proliferation and viability as described in the Materials and Methods and reported here as percent of the untreated control. Values for HPBMC treated with ovine IFN τ and IFN τ /IFN α chimeric were not significantly different from the untreated control whereas values for HPBMC treated with human IFN α were significantly different ($p < 0.05$) from the untreated controls as determined using the Wilcoxon signed-rank test.

Detailed Description of the Invention

The subject invention concerns novel therapeutic and prophylactic methods for treating any condition where suppression or inhibition of IgE production is useful or beneficial, including allergic diseases and other IgE-related diseases or conditions. Disease conditions that can be treated according to the subject methods include, but are not limited to, allergic rhinitis, atopic dermatitis, bronchial asthma and food allergy. In the methods of the present invention, an effective amount of a composition comprising a type I IFN, such as IFN α , IFN β , IFN τ or IFN ω , or a chimeric IFN, is administered to a person having a condition where suppression or inhibition of IgE production is clinically desirable.

In one embodiment of the subject invention, an effective amount of IFN τ is administered to a person or animal afflicted with, or predisposed to, an allergic condition or other IgE-associated condition. The IFN used in the subject methods can be from any animal that produces the IFN, including but not limited to, primate, ovine, bovine and others.

In another embodiment of the subject invention, a mammalian IFN that has an amino acid sequence that provides the low toxicity of IFN τ with the bioactivity of other type I IFNs is used in the subject methods to treat a person or animal afflicted with, or predisposed to, an allergic condition or other conditions or diseases where suppression of IgE production or response is beneficial. In a preferred embodiment, an effective amount of a chimeric IFN comprising a mammalian IFN τ amino terminus and a human type I IFN carboxy terminus, such as that from IFN α , is administered to a person afflicted with, or predisposed to, an

allergic condition or other IgE-associated condition. More preferably, the chimeric IFN protein comprises amino acid residues 1-27 of ovine IFN τ and amino acid residues 28-166 of human IFN α . In an exemplified embodiment, the IFN α is IFN α D.

5 The subject invention also concerns methods for suppressing IgE production and cell proliferation *in vivo* and *in vitro* using a type I interferon. As exemplified herein, splenocytes taken from an ovalbumin (OVA) immunized animal using either IFN τ or a chimeric IFN τ protein suppressed OVA-induced proliferation and IgE production. Thus, the methods of the subject invention can be used to suppress IgE production in an animal or person. The methods of the subject invention can also be used to suppress IgE production
10 *in vitro*.

The present invention also concerns methods for inhibiting B cell and T cell responses, including cell proliferation and cytokine production. As exemplified herein, type I IFNs can be used to inhibit production of IL-4. The cytokine IL-4 plays a central role in isotype switching of the B cells to IgE production.

15 Biologically active muteins (mutated proteins) of the subject polypeptides, as well as other molecules, such as fragments, peptides and variants, that possess substantially the same IgE-suppressive bioactivity as the subject IFN polypeptides, are contemplated within the scope of the subject methods. For example, IFN τ polypeptides that contain amino acid substitutions, insertions, or deletions that do not substantially decrease the biological activity and function of the mutant polypeptide in comparison to native polypeptide are within the
20 scope of the present invention. Specifically contemplated within the scope of the invention are fragments of the type I IFN that retain substantially the same biological activity as the full length IFN. The muteins and fragments of IFNs can be readily produced using standard methods known in the art. For example, by using the Bal31 exonuclease (Wei *et al.*, 1983),
25 the skilled artisan can systematically remove nucleotides from either or both ends of the polynucleotide to generate a spectrum of polynucleotide fragments that when expressed provide the IFN fragment encoded by the polynucleotide.

Therapeutic application of the subject polypeptides and compositions containing them can be accomplished by any suitable therapeutic method and technique presently or prospectively known to those skilled in the art. The polypeptides can be administered by any suitable route known in the art including, for example, oral, parenteral, subcutaneous, or intravenous routes of administration. Administration of the polypeptides of the invention can be continuous or at distinct intervals as can be readily determined by a person skilled in the art.

The subject invention also concerns chimeric IFN polypeptides and the polynucleotides that encode them. In one embodiment, the chimeric IFNs comprise ovine and human IFN regions. Preferably, a chimeric IFN protein of the invention comprises an ovine IFN τ amino terminus and a human IFN α carboxy terminus. In an exemplified embodiment, the chimeric IFN protein comprises amino acid residues 1-27 of ovine IFN τ and residues 28-166 of human IFN α D. The polynucleotide sequences encoding the chimeric IFNs of the present invention can be readily constructed by those skilled in the art having the knowledge of the amino acid sequences of the subject polypeptides. As would be appreciated by one skilled in the art, a number of different polynucleotide sequences can be constructed due to the degeneracy of the genetic code. The choice of a particular nucleotide sequence could depend, for example, upon the codon usage of a particular expression system.

Compounds useful in the subject invention can be formulated according to known methods for preparing pharmaceutically useful compositions. Formulations are described in detail in a number of sources which are well known and readily available to those skilled in the art. For example, *Remington's Pharmaceutical Science* by E.W. Martin describes formulations which can be used in connection with the subject invention. In general, the compositions of the subject invention will be formulated such that an effective amount of the bioactive polypeptide is combined with a suitable carrier in order to facilitate effective administration of the composition. The compositions used in the present methods can also be in a variety of forms. These include, for example, solid, semi-solid, and liquid dosage forms, such as tablets, pills, powders, liquid solutions or suspension, suppositories, injectable

and infusible solutions, and sprays. The preferred form depends on the intended mode of administration and therapeutic application. The compositions also preferably include conventional pharmaceutically acceptable carriers and diluents which are known to those skilled in the art.

5 The compounds of the subject invention can also be administered utilizing liposome technology, slow release capsules, implantable pumps, and biodegradable containers. These delivery methods can, advantageously, provide a uniform dosage over an extended period of time.

10 Examples of carriers or diluents for use with the subject polypeptides include ethanol, dimethyl sulfoxide, glycerol, alumina, starch, and equivalent carriers and diluents. To provide for the administration of such dosages for the desired therapeutic treatment, new pharmaceutical compositions of the invention will advantageously comprise between about 0.1% and 45%, and especially, 1 and 15% by weight of the total of one or more of the polypeptides based on the weight of the total composition including carrier or diluent.

15 All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety to the extent they are not inconsistent with the explicit teachings of this specification.

Materials and Methods

Interferons.

20

25 The ovine interferon tau (IFN τ) gene was expressed in *Pichia pastoris* using a synthetic gene construct (Heeke *et al.*, 1996). IFN τ was secreted into the medium and was purified by successive DEAE-cellulose and hydroxylapatite chromatography to electrophoretic homogeneity as determined by SDS-PAGE and silver staining analysis. The purified protein had a specific activity of $2.9 - 4.4 \times 10^7$ U/mg protein as measured by antiviral activity using a standard viral microplaque reduction assay on MDBK (Pontzer *et al.*, 1991). The recombinant human IFN α D was from Biosource International, Camarillo, CA. The "humanized" IFN τ /IFN α D chimeric protein was constructed using residues 1-27

of the ovine IFN τ and residues 28-166 of the human IFN α D and was expressed in *Pichia pastoris* as previously described for ovine IFN τ (Heeke *et al.*, 1996).

IFN τ was administered intraperitoneally (ip) at 5×10^5 U/mouse daily starting 96 h prior to immunization and continuing everyday thereafter for a month. Control mice received PBS.

Immunization of mice.

BALB/C mice were immunized ip with 10 μ g of ovalbumin (OVA) (Sigma, St. Louis, MO) precipitated with 5 mg aluminum hydroxide gel in a total volume of 100 μ L. Aluminum hydroxide gel was prepared as previously described (Revoltella *et al.*, 1969; Warner *et al.*, 1968). Mice were immunized again 7 days after the initial immunization using the same protocol. The mice were exposed to aerosolized OVA from 1% OVA (w/v) in PBS on days 19 and 20 after immunization. Aerosolization was performed for 20 min using the Pari Jet + nebulizer and compressor (Pari Respiratory Equipment, Inc., Midlothian, Virginia). Mice were housed and cared for at the Animal Resource Center (University of Florida), and all experimental animal uses were approved by the Institutional Animal Care and Use Committee (IACUC).

Histological evaluation.

The lungs of OVA-immunized mice that had been exposed to aerosolized OVA were intratracheally perfused with 4% paraformaldehyde solution. The lungs were fixed for 2-3 days in the same solution after which lung samples were embedded in paraffin and sectioned. Samples were then stained with hematoxylin and eosin. Also, blood smears were prepared on slides from the same mice, and slides were stained with the "LEUKOSTAT" staining kit (Fisher Scientific, Pittsburgh, PA) for the determination of differential white blood cell count. A total of 150 white blood cells were evaluated.

Proliferation assay.

Spleen cells taken from mice 21 days after immunization from PBS or IFN τ -treated mice were cultured at 5×10^5 cell/well in presence of OVA for 72 to 84 h in RPMI 1640 medium containing 10% FBS. In other assays, PBS-treated mouse splenocytes were incubated at 5×10^5 cells/well in presence of OVA and various IFNs (10,000 to 15,000 U/ml) for 72 to 84 h. The cultures were pulsed with [3 H]-thymidine (1.0 uCi/well; Amersham, Indianapolis, IN) and harvested 12 h later on to filter paper discs using a cell harvester. Cell associated radioactivity was quantified using a β -scintillation counter and activity reported in CPM. Proliferation assays on the U266BL myeloma B cells were also carried out by incubating the cells in RPMI 1640 medium overnight prior to culturing 4×10^5 cells/well with various IFNs at 10,000 to 15,000 U/ml in RPMI 1640 containing 4% FBS. Cultures were incubated for 72-84 h after which cells were pulsed with [3 H]-thymidine prior to harvest 12 h later. Cell associated radioactivity was quantified using β -scintillation counter and activity reported in CPM. The U266BL cell line, an IgE producing myeloma that was isolated from the peripheral blood of a patient, provides a system to assess the direct effects of IFN τ on an IgE producing cell (Nilsson *et al.*, 1970). This cell line allows one to study the effects of IFN τ on B cells with ongoing IgE synthesis.

Enzyme Linked Immunoabsorbent Assay.

OVA was resuspended in binding buffer (0.1 M carbonate/bicarbonate, pH 9.6) and absorbed onto the flat bottoms of plastic 96-well tissue culture wells overnight at 4°C at a concentration of 2 μ g/well and subsequently evaporated to dryness. The plates were treated with blocking buffer, 5% powdered milk in PBS, for 2 h in order to block nonspecific binding and then washed three times with PBS containing 0.05% Tween 20. Various dilutions of sera from BALB/C mice which were IFN τ -treated or PBS-treated or nonimmunized (naive) mice were added to the wells and incubated for 3 h at room temperature. After extensive washing, rabbit anti-mouse IgE antibody (Accurate, NY) was added. Plates were washed three times prior to addition of 1:1000 dilution of horse radish

peroxide (HRP) conjugated goat anti-rabbit immunoglobulin (Amersham Pharmacia Biotech, Piscataway NJ). Color development was monitored at 490 nm in an ELISA plate reader (BioRad, Richmond, CA) after the substrate solution (0.002M o-phenylenediamine dihydrochloride, 0.012% H₂O₂, 0.05 M Na Citrate, 0.05 M citrate) was added and the reaction terminated with 2M H₂SO₄.

For the detection of IL-4 in blood, sera samples were collected from PBS- or IFN τ treated mice and incubated in 96 well plates that had rabbit polyclonal anti-mouse IL-4 antibody (Biosource Int., Camarillo, CA) bound to it. After washing, 25 μ g/ml of rat monoclonal anti-mouse IL-4 biotinylated antibody was added for 1 h incubation. A 1:1000 dilution of HRP-conjugated avidin was added after the incubation and washings, and substrate color development was monitored as described above. The limit of detection of the IL-4 ELISA was 7 ng/ml.

Western blot.

Culture supernatants from both IFN or media treated OVA-sensitized splenocytes, and U266BL myeloma B-cells, were loaded at 10 μ g/lane (total protein) on 15% and 10% SDS-PAGE READY GELS (BioRad, Richmond, CA) respectively, and run at 100 volts. Overnight transfer onto nitrocellulose membrane was carried out, after which the membrane was blocked with 5% milk in Tris-buffered saline pH 7.5, 0.1% Tween 20 for 1 h. Immunoblots were incubated with a 1:1000 dilution of either goat anti-human IgE (Biosource Int., Camarillo, CA) coupled to HRP or rabbit anti-mouse IgE (Accurate, NY). The mouse IgE blot was incubated further after three washes with HRP conjugated anti-rabbit Ig (Amersham Pharmacia Biotech, Piscataway, NY) for 1 h. Blots were washed and analyzed through film development.

Toxicity Assays of IFNs.

Toxicity assays using human peripheral blood mononuclear cells (HPBMC) were carried out by culturing HPBMC in the presence of varying concentrations of IFN τ , IFN α D,

and chimeric IFN τ /IFN α D for seven days. Metabolic activity of HPBMC was assessed using WST-1 (Boehringer-Mannheim, Indianapolis, IN), which measures cell proliferation and viability based on the enzymatic activity of mitochondrial dehydrogenases in viable cells.

5

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

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Example 1 - IFN τ inhibits production of OVA-specific IgE antibody in mice

As shown in Figure 1A, daily injections (ip) of IFN τ at 5×10^5 units beginning prior to OVA-aluminum hydroxide injection (ip) blocked IgE antibody production by over 50%. Further, the blocking continued even when the mice were challenged with aerosolized OVA (Figure 1B). Thus, IFN τ inhibited OVA-specific IgE antibody production under conditions where the mice were immunized to OVA by injection and challenged by inhalation.

15

Example 2 - Reduced inflammatory cell infiltration of IFN τ -treated mice

OVA immunized mice were challenged with aerosolized OVA following treatment with IFN τ in order to determine if the IFN treatment inhibited inflammatory cell infiltration into the lungs. IFN τ inhibition of cellular infiltration is shown in Figure 2 where lung sections of naive (Figure 2A), PBS treated (Figure 2B), and IFN τ treated (Figure 2C) mice are compared. The destruction of the integrity of the epithelial tissue lining the bronchiole of the PBS treated mice (Figure 2B) versus the protection in IFN τ treated mice (Figure 2C) was evident. Eosinophil, basophil, and lymphocytic infiltration was assessed around bronchioles and blood vessels of IFN τ and PBS treated (control) mice. Significantly fewer bronchioles had peribronchiolar aggregates of granulocytes with IFN τ treatment (20%) as compared to PBS treatment (64%) (Table I).

20

25

Table I. IFN τ reduces OVA-induced inflammatory cell infiltration into the bronchioles*

Treatment	Lung sections	
	Granulocyte aggregates (% Airways)	Lymphocyte aggregates (% Airways)
PBS	64 \pm 6.0	78 \pm 7.0
IFN τ	20 \pm 7.0	34 \pm 3.0
Naive	0	0

Treatment	Blood smears				
	Eosinophil (%)	Basophil (%)	Neutrophil (%)	Monocyte (%)	Lymphocyte (%)
PBS	11 \pm 5.0	6.0 \pm 1.0	20 \pm 0.7	10 \pm 1.4	58 \pm 6.0
IFN τ	2.5 \pm 0.7	1.5 \pm 0.7	15 \pm 1.4	5.0 \pm 1.4	72 \pm 8.0
Naive	0.2 \pm 0.3	0.5 \pm 0.7	19 \pm 1.4	10 \pm 0.7	71 \pm 4.0

*Mice were treated (ip) with 5×10^5 U of IFN τ daily starting three days prior to OVA immunization as described in the Materials and Methods section. Airways having peribronchiolar aggregates were enumerated and divided by the total number of bronchioles examined in each section. Differential white cell counts from blood smears are presented as percent cell type. Statistical significance for the inhibition of granulocytes and lymphocyte aggregates around the bronchioles was shown by χ^2 test for IFN τ treatment as compared to PBS treatment ($p < 0.001$). Statistical significance for the inhibition of eosinophil ($p < 0.05$) and basophil ($p < 0.1$) by IFN τ treatment as compared to PBS treatment was shown by Student's t test.

Also, 78% of lung bronchioles in PBS-treated mice had lymphocytic infiltration compared to 34% for IFN τ treatment. In addition, differential counts on the blood of mice showed that IFN τ -treated mice had lower levels of eosinophils and basophils as compared to those of PBS-treated groups, while neutrophil, monocyte, and lymphocyte levels were not significantly different from either the PBS-treated or naive (nonimmunized) mice. Thus, the data show that IFN τ treatment inhibits inflammatory cell infiltration into the lungs of OVA-sensitized- mice when exposed to aerosolized OVA allergen.

Example 3 - IFN τ -treated mice have lower IL-4 levels than control mice

IL-4 levels in sera of mice treated with PBS, IFN τ , or nonimmunized (naive) mice were measured after aerosolized OVA exposure, which was given 20 days after immunization. It has been shown previously that IL-4 may be necessary for inducing the IgE isotype class switch in B cells (Lanzavecchia *et al.*, 1984; Coffmann, Carty *et al.*, 1986; Coffmann, Ohara *et al.*, 1986; Rothman *et al.*, 1988). As shown in Figure 3, IL-4 levels in the IFN τ -treated group were less than half of those of the PBS-treated group.

Example 4 - *In vivo* IFN τ -treatment inhibits OVA-specific splenocyte proliferation

Spleens from nonimmunized (naive) mice and PBS- or IFN τ -treated OVA-immunized mice were removed 20 days after OVA immunization in order to determine the inhibitory effect of IFN τ treatment on OVA induced proliferation. Splenocytes were incubated in the presence of OVA for 84 h after which proliferation was assessed. As shown in Figure 4, significantly reduced proliferation in response to OVA was observed in splenocytes from IFN τ -treated mice as compared to PBS-treated control mice. This proliferative activity was specific for OVA since bovine serum albumin (BSA) and myelin basic protein (MBP) did not activate splenocytes (Figure 4 inset). Thus, *in vivo* IFN τ treatment of allergen-primed mice inhibited cellular proliferation in response to allergen.

Example 5 - *In vitro* IFN treatment of OVA-sensitized splenocytes inhibits OVA-specific splenocytes proliferation

OVA sensitized splenocytes were treated *in vitro* with various IFNs in order to determine their effect on previously sensitized cells. Spleens were removed 20 days after OVA immunization and after aerosolized OVA treatment, and cultured with various type I IFNs for 84 h after which proliferation was assessed. In addition to ovine IFN τ treatment, human IFN α D, and chimeric IFN τ /IFN α D were also tested for their effects on OVA induced proliferation. The chimeric was tested as a potential for "humanized" IFN τ for possible human therapy. As shown in Figure 5, both IFN τ and IFN α D inhibited OVA-specific

splenocyte proliferation. Furthermore, the IFN τ /IFN α D chimeric, which contained amino acid residues 1-27 of ovine IFN τ and residues 28-166 of human IFN α D, also had an inhibitory effect. Thus, treatment of OVA-sensitized splenocytes *in vitro* with type I IFNs suppressed cell proliferation.

Example 6 - Type I IFNs inhibit mouse and human IgE production

Immunoblots for the detection of IgE antibodies were performed on culture supernatants taken from the proliferation assay experiments performed in Figure 4 and 5. As shown in Figure 6A, IgE was detected in cultures containing PBS-treated splenocytes that were incubated in the presence of OVA. There was little or no IgE in supernatants from splenocytes of IFN τ -treated mice. Immunoblots for detection of IgE in culture supernatants from *in vitro* IFN treatment of OVA sensitized mouse splenocytes showed an inhibition of IgE production by all of the type I IFNs as compared to the media control (Figure 6B). The chimeric IFN τ /IFN α D protein and the IFN α D protein were better inhibitors than was IFN τ , however.

The U266BL human myeloma cell line, which produces IgE antibodies constitutively was also incubated with the type I IFNs in order to determine if the IFNs had a direct effect on the human IgE-producing B cells. Cells were starved overnight prior to treatment with various type I IFNs, including the IFN τ /IFN α D chimeric. After incubation with the IFNs for 96 h, supernatants were collected and IgE levels were detected by immunoblot. As shown in Figure 6C, IgE levels were lower in the IFN treated groups as compared to the media control. Thus, type I IFNs inhibit U266BL human myeloma cells and OVA-specific mouse B cells from producing IgE antibodies.

Example 7 - IFN inhibition of proliferation of the human IgE-producing myeloma cells

The U266BL myeloma cells were cultured in the presence of 10,000 U/ml of IFNs for 72 h, after which proliferation was measured. All IFNs inhibited proliferation of the cells by about 50% or more with IFN α D being the most effective and IFN τ being the least

effective (Figure 7). Viabilities were determined and showed that the IFN α D was the most toxic (67% viability) as compared to the IFN τ (80% viability) and the IFN τ /IFN α chimeric (75% viability). The IFN τ /IFN α D chimeric suppressed proliferation more effectively than IFN τ but not as effectively as IFN α D. Thus, type I IFNs, with various toxicity levels, inhibit the cell proliferation of the human IgE producing cell line, U266BL.

Example 8 - Lack of toxicity of the IFN τ /IFN α D chimeric on human peripheral blood mononuclear cells (HPBMC)

The IFN τ /IFN α D chimeric was compared with recombinant ovine IFN τ and recombinant human IFN α D for toxicity on HPBMC. After seven days of treatment of the HPBMC with various concentrations of IFNs (250 to 100,000 U/ml), toxicities were measured based on the enzymatic activity of the mitochondrial dehydrogenases in viable cells. As shown in Figure 8, human IFN α D was toxic at concentrations of 1,000 to 100,000 U/ml as compared to ovine IFN τ and IFN τ /IFN α D chimeric, which did not show toxicity at any concentration. Thus, the IFN τ /IFN α D chimeric, like ovine IFN τ , lacked the toxicity associated with human IFN α D.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

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Claims

We claim:

1 1. A method for suppressing or inhibiting IgE production, said method comprising
2 administering an effective amount of a type I interferon, or a biologically active mutein,
3 fragment, variant or peptide thereof.

1 2. The method according to claim 1, wherein said type I interferon is selected from
2 the group consisting of IFN α , IFN β , IFN τ and IFN ω .

1 3. The method according to claim 2, wherein said type I interferon is IFN τ .

1 4. The method according to claim 1, wherein said type I interferon is a chimeric IFN
2 comprising part of at least two IFNs selected from the group consisting of IFN α , IFN β , IFN τ
3 and IFN ω .

1 5. The method according to claim 4, wherein said chimeric IFN comprises a
2 mammalian IFN τ amino terminus and a human type I IFN carboxy terminus other than IFN τ .

1 6. The method according to claim 5, wherein said mammalian IFN τ amino terminus
2 is from a mammal selected from the group consisting of primate, ovine and bovine.

1 7. The method according to claim 5, wherein said chimeric IFN comprises amino
2 acid residues from about 1 to about 27 of ovine IFN τ and amino acid residues from about 28
3 to about 166 of human IFN α .

1 8. The method according to claim 7, wherein said IFN α is IFN α D.

1 9. The method according to claim 1, wherein said type I interferon is administered
2 to a person or animal in need of suppression or inhibition of IgE production.

1 10. The method according to claim 1, wherein said suppression or inhibition of IgE
2 production occurs through inhibition of B-cell IgE secretion or inhibition of B-cell
3 proliferation.

1 11. The method according to claim 9, wherein said type I interferon is administered
2 by routes selected from the group consisting of oral administration, parenteral administration,
3 subcutaneous administration and intravenous administration.

1 12. The method according to claim 11, wherein said person or animal is afflicted
2 with, or predisposed to, an IgE-related condition.

1 13. The method according to claim 12, wherein said IgE-related condition is an
2 allergic condition selected from the group consisting of allergic rhinitis, atopic dermatitis,
3 bronchial asthma and food allergy.

1 14. The method according to claim 1, wherein said type I interferon is administered
2 *in vitro*.

1 15. The method according to claim 1, wherein said type I interferon is formulated
2 in a pharmaceutically acceptable carrier or diluent.

1 16. A composition comprising a chimeric type I interferon, or a biologically active
2 mutein, fragment, variant or peptide thereof, which is capable of suppressing or inhibiting

IgE production, wherein said chimeric IFN comprises part of at least two IFNs selected from the group consisting of IFN α , IFN β , IFN τ and IFN ω .

17. The composition according to claim 16, wherein said suppression or inhibition of IgE production occurs through inhibition of B-cell IgE secretion or inhibition of B-cell proliferation.

18. The composition according to claim 16, wherein said chimeric IFN comprises a mammalian IFN τ amino terminus and a human type I IFN carboxy terminus other than IFN τ .

19. The composition according to claim 18, wherein said mammalian IFN τ amino terminus is from a mammal selected from the group consisting of primate, ovine and bovine.

20. The composition according to claim 18, wherein said chimeric IFN comprises amino acid residues from about 1 to about 27 of ovine IFN τ and amino acid residues from about 28 to about 166 of human IFN α .

21. The composition according to claim 20, wherein said IFN α is IFN α D.

22. The composition according to claim 16, wherein said chimeric IFN is recombinantly produced and is expressed in *Pichia pastoris*.

23. A polynucleotide that encodes the chimeric IFN of claim 16.

24. A method for suppressing or inhibiting IL-4 production, said method comprising contacting an immune cell with a type I interferon, or a biologically active mutein, fragment, variant or peptide thereof.

Abstract

5 The subject invention concerns novel methods and materials for treating patients afflicted with allergic conditions, such as allergic rhinitis, atopic dermatitis, bronchial asthma and food allergy. The method of the subject invention comprises administering interferon tau (IFN τ) or a chimeric IFN (ovine IFN τ (1-27)/human IFN α D (28-166)) to a person afflicted with an allergic condition. When administered, IFN τ and chimeric IFN suppress the production of IgE antibodies without toxic side effects. The subject invention also concerns chimeric ovine/human IFNs which can be used in the methods of the invention.

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FIG. 1A

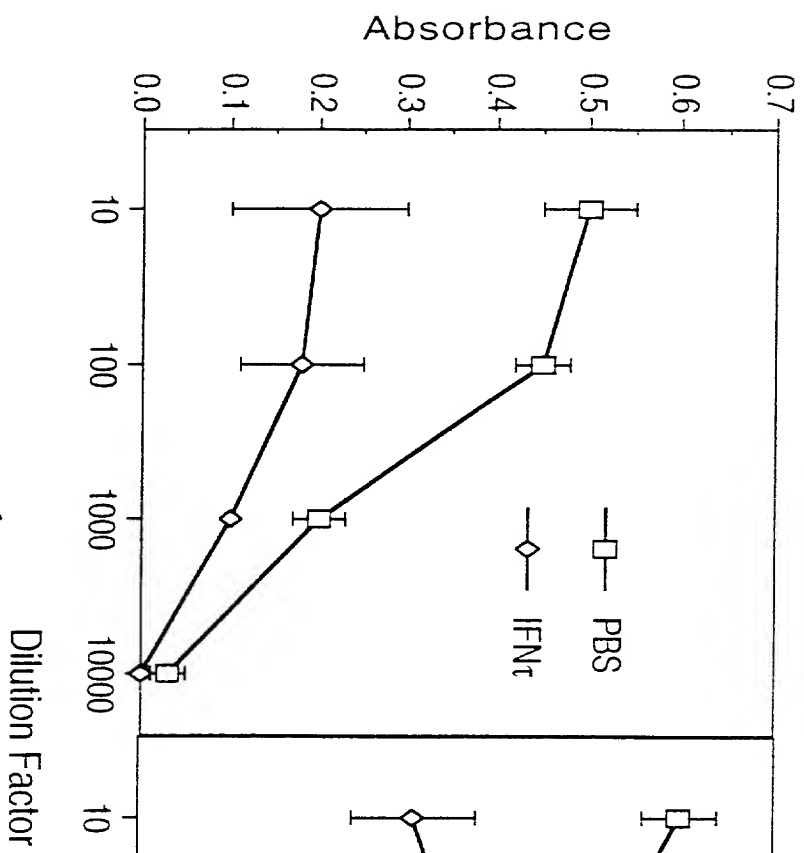


FIG. 1B

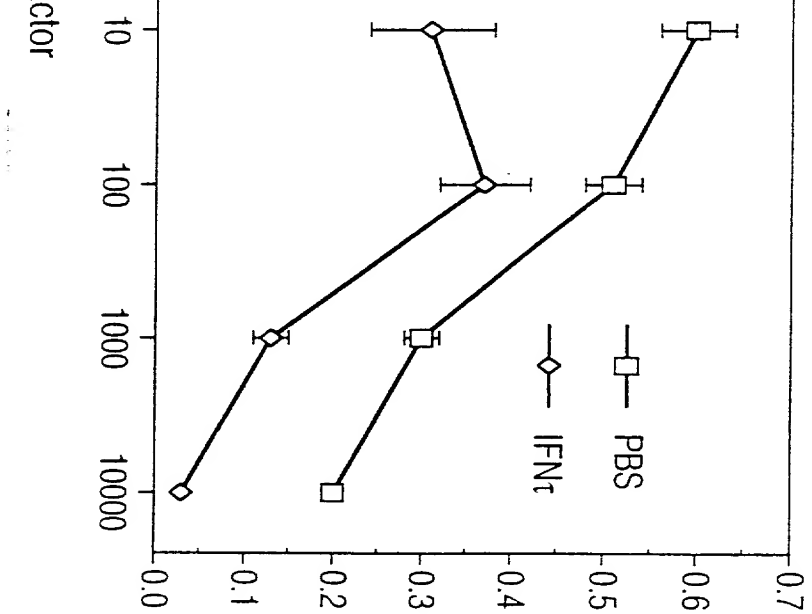


FIG. 2A



FIG. 2B

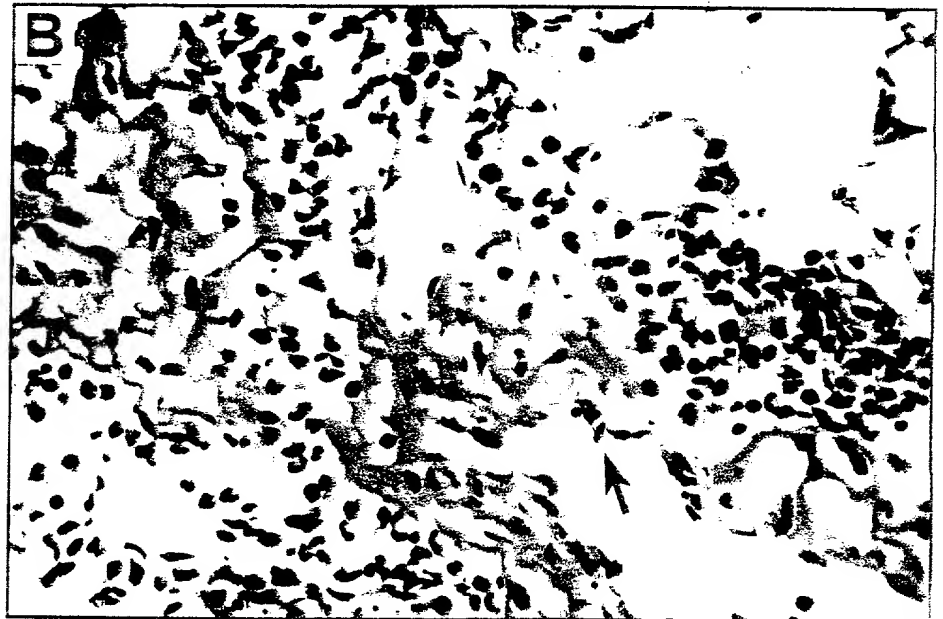
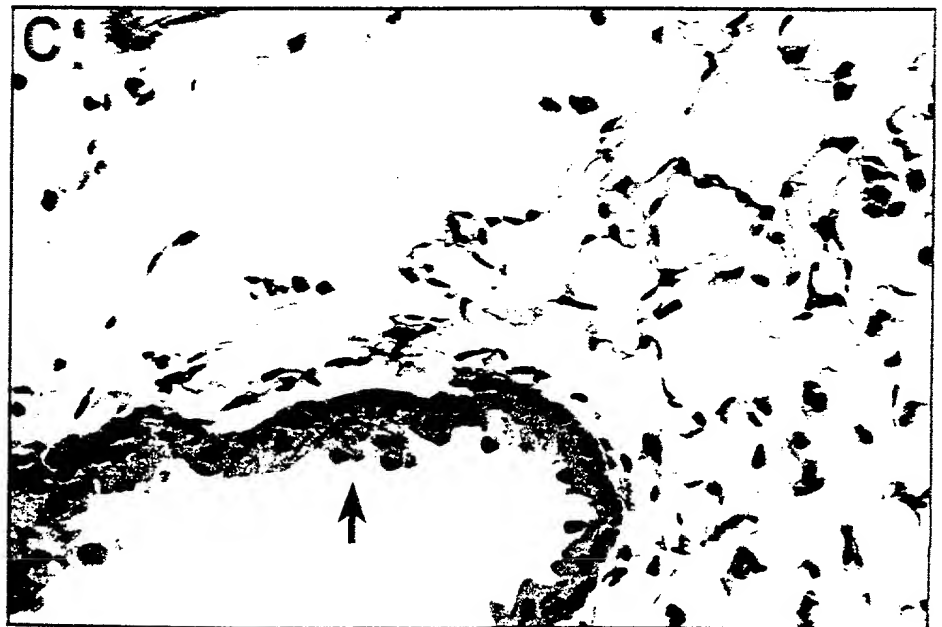


FIG. 2C



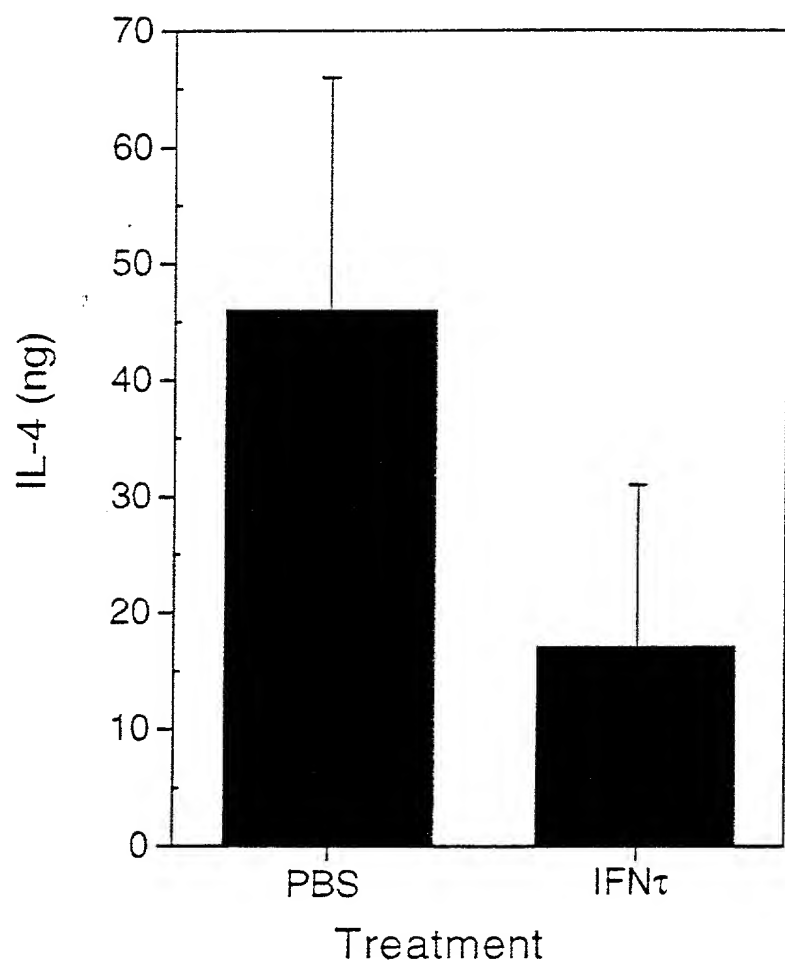


FIG. 3

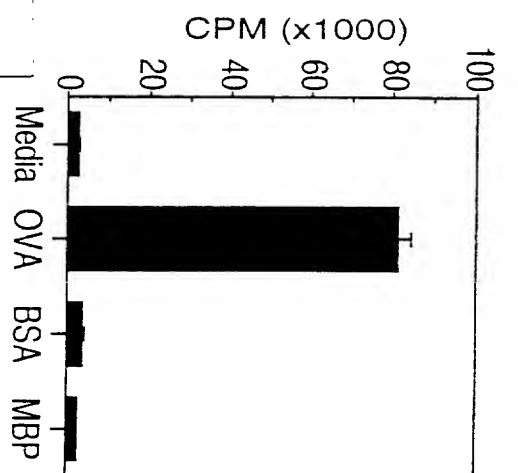


FIG. 4B

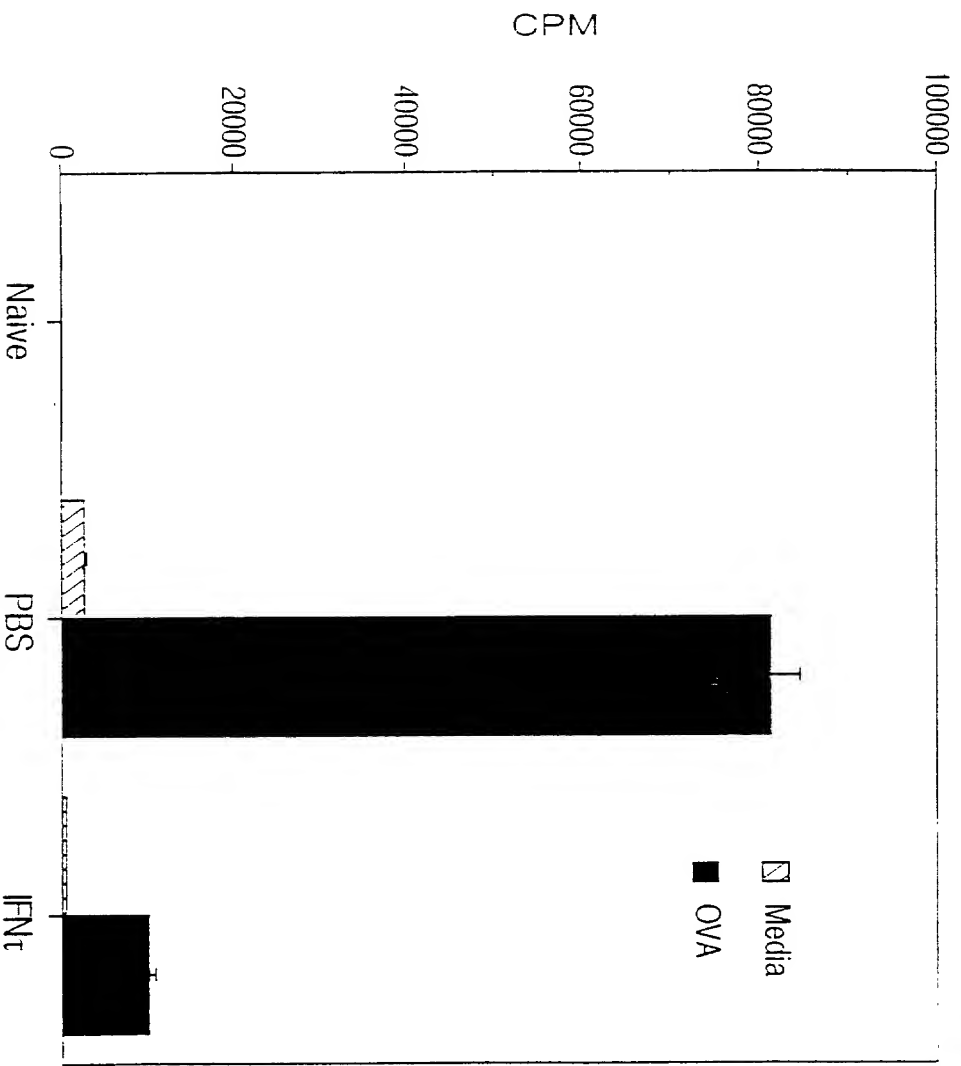


FIG. 4A *SI* MCS

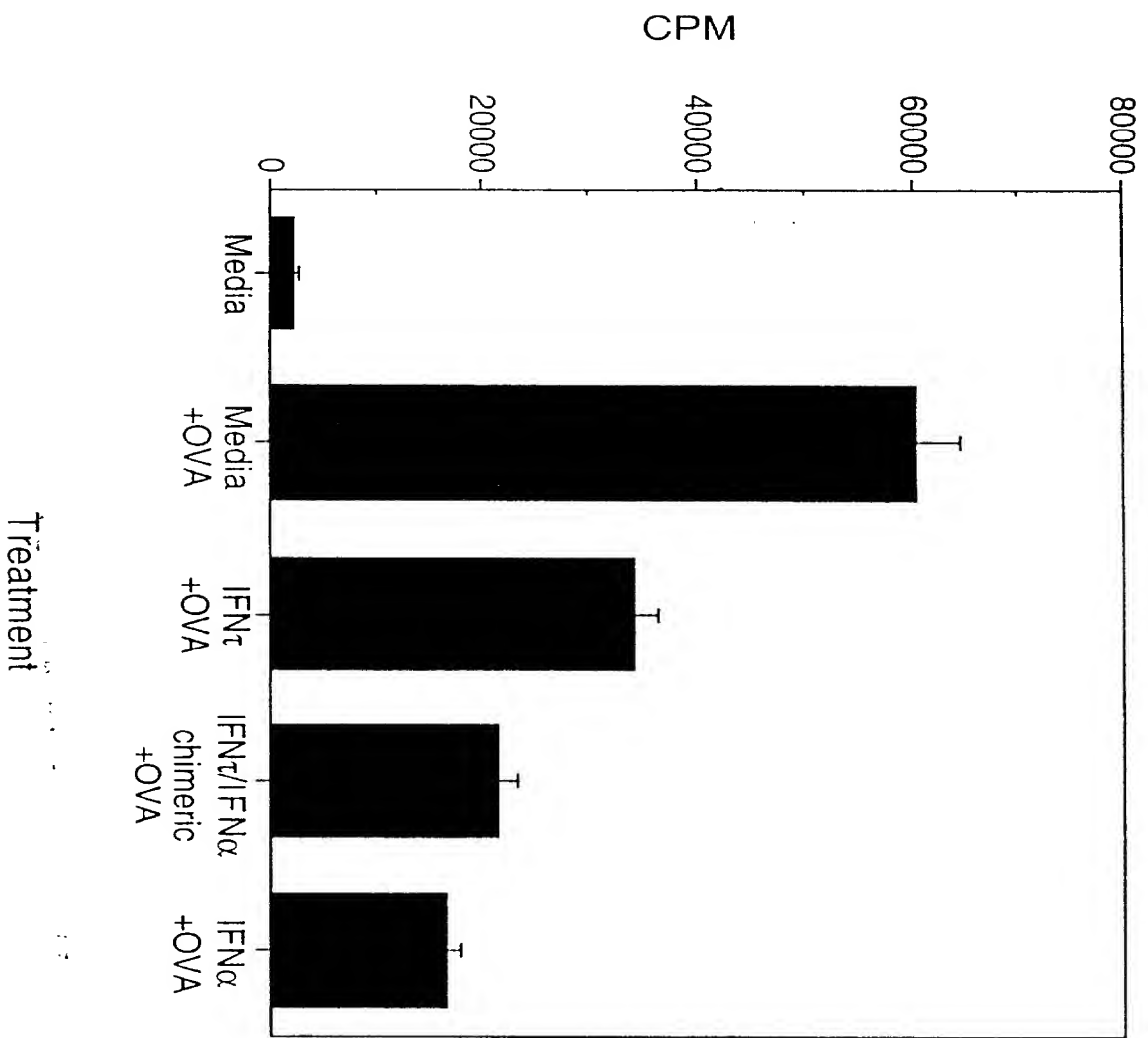


FIG. 5 27 WCS

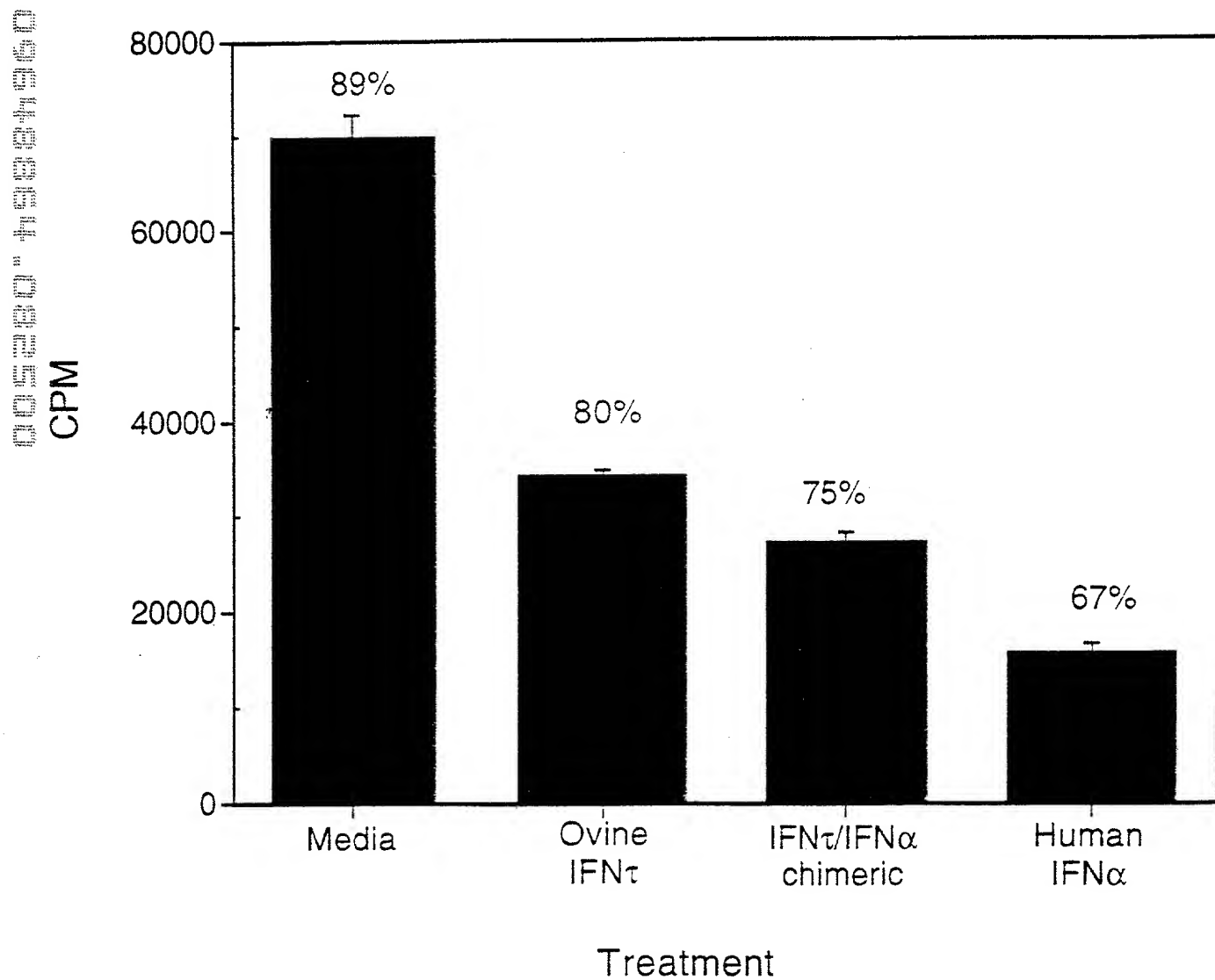
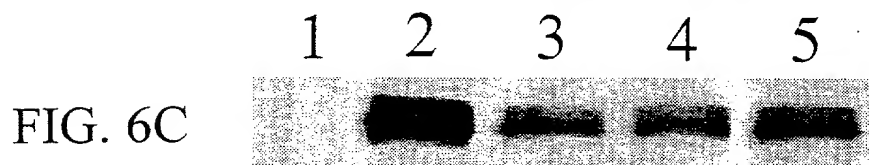
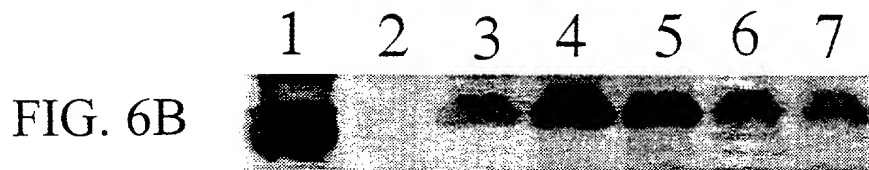
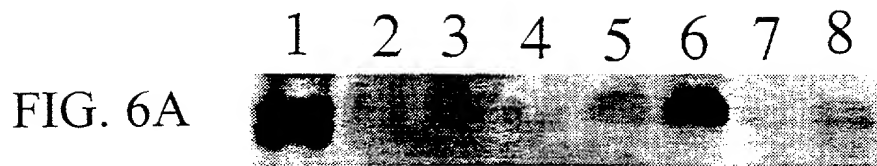
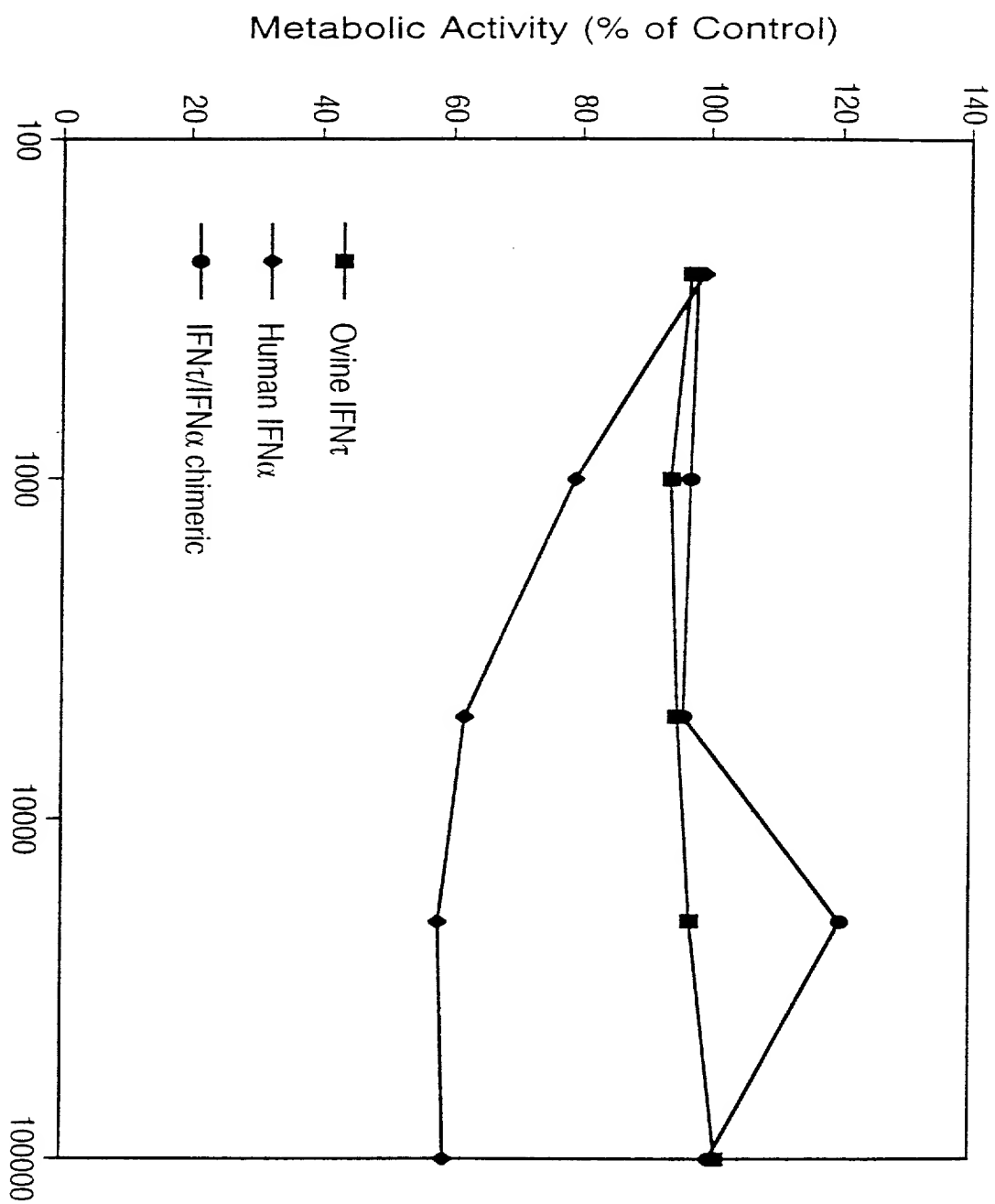


FIG. 7



IFN (U/ml)
21 NCS
FIG. 8

DECLARATION (37 CFR 1.63) AND POWER OF ATTORNEY

As a below-named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name; and

I believe that I am the original, first, and sole inventor (if only one name is listed below), or an original, first, and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **MATERIALS AND METHODS FOR INHIBITION OF IgE PRODUCTION** the specification for which

☒ is attached hereto.

☐ was filed _____, Serial No. _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code §119 and/or §365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Application Serial No.	Country	Filing Date	Priority Claimed
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I hereby claim priority benefits under Title 35, United States Code §119 of any provisional application(s) for patent listed below:

Application Serial No.	Filing Date	Priority Claimed
60/151,026	August 27, 1999	Yes

I hereby claim the benefit under Title 35, United States Code, §120 and/or §365 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status (patented, pending, abandoned)
---------------------------	-------------	--

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following persons registered to practice before the Patent and Trademark Office as my attorneys with full power of substitution and revocation to prosecute this application and all divisions and continuations thereof and to transact all business in the Patent and Trademark Office connected therewith: David R. Saliwanchik, Reg. No. 31,794; Jeff Lloyd, Reg. No. 35,589; Doran R. Pace, Reg. No. 38,261; Christine Q. McLeod, Reg. No. 36,213; Jay M. Sanders, Reg. No. 39,355; James S. Parker, Reg. No. 40,119; Jean Kyle, Reg. No. 36,987; Frank C. Eisenschenk, Reg. No. 45,332; Seth M. Blum, Reg. No. 45,489; Glenn P. Ladwig, Reg. No. P-46,853.

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Residence _____ Citizenship _____

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Signature of Third Joint Inventor

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Residence _____ Citizenship _____

Post Office Address _____

Date _____

Signature of Fourth Joint Inventor

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